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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Mary J. Laughlin
Serial No.	:	10/730,549
Filing Date	:	December 5, 2003
For	:	CELL-BASED THERAPIES FOR ISCHEMIA
Group Art Unit	:	1651
Examiner	:	Lora Elizabeth Barnhart
Attorney Docket No.	:	CWR-019292US ORD

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR §1.132

Sir:

I, Mary Laughlin M.D. declare as follows:

1. I am a co-inventor of the invention entitled "Cell-Based Therapies for Ischemia", disclosed and claimed in U.S. Application Serial No. 10/754,102 filed December 5, 2003, which claims priority from U.S. Provisional Patent Application 60/431,347, filed December 5, 2002.

2. I obtained my Bachelor of Science Nursing from University of Rochester, NY, my Master of Science Nurse Anesthesia, from State University of

New York at Buffalo, NY, my Doctor of Medicine, from State University of New York at Buffalo, NY.

3. I was a Medical Intern, from1988-1989 at Duke University Medical Center, NC, a Resident in Internal Medicine, from 1989-1991 at Duke University Medical Center, NC, a Clinical Research Fellow in Hematology/Oncology, from 1991-1992, at Duke University Medical Center, a Clinical Research Fellow in Hematology/Oncology/Bone Marrow Transplantation, from 1992-1994, at Roswell Park Cancer Institute, NY, an Assistant Professor of Medicine, from 1994-1998, at Duke University Medical Center, NC, an Assistant Professor of Medicine, from 1998-2002, at Case Western Reserve University, OH, an associate Professor of Medicine, from 2003-2010, at Case Western Reserve University, OH, and a Professor of Medicine and Section Head of Stem Cell Transplantation, from 2010 to the present, at University of Virginia, VA. I have received numerous national grants with respect to stem cells and umbilical cord blood-derived endothelial precursor cells and have published extensively on stem cell transplantation and umbilical cord blood-derived endothelial precursor cells, as indicated in my attached *curriculum vitae*, which is provided herewith as Attachment A.

3. As described in Example 11 of the patent application, I performed a series of experiments to determine whether stromal cells added to umbilical cord blood (UCB)-derived endothelial progenitor cells (EPCs) would augment neovascularization *in vivo* in a mouse hindlimb ischemia model. In these experiments human mesenchymal stem cells (hMSCs) were isolated and culture expanded from bone marrow aspirates as described in Example 6, and CD133+

endothelial precursor cells were isolated and cultured expanded from UCB as described in Example 3 and Example 4. $1x10^6$ CD133+ cells and $1x10^6$ hMSC were co-injected intracardially into mice that had undergone hind-limb femoral artery ligation by the method described in Example 2. Blood flow was measured by laser Doppler flowmeter over time, and the results, which are illustrated in FIG. 17, are expressed as the ratio between the blood flow in the injured and the uninjured leg over time. The results show increased blood flow in the mouse receiving both CD133+ cells and hMSCs at day 7 after surgery compared with mice infused with CD133+ cells or hMSCs alone. This result suggests that improved blood flow was achieved at an earlier time point (day 7) after co-infusion of hMSCs with CD133+ cells compared to infusion with CD133+ cells or hMSCs alone.

4. Additional experiments conducted in my laboratory tested the effectiveness human bone marrow (BM)-derived mesenchymal stem cells (MSCs) injected alone and in combination with UCB-derived $CD133^+$ HSCs in treating an *in vivo* mouse hindlimb femoral ligation model. Significantly enhanced blood flow and histologic evidence of angiogenesis was noted in an *in vivo* non-obese diabetic/severe combined immunodeficiency disease (NOD/SCID) mouse hindlimb femoral ligation model (See Attachment B). Femoral ligation and resection was performed and study animals were randomized to one of three treatment groups. Group 1, control, was treated with injection of media (0.02 ml). Group 2 animals received third passage human MSC ($1x10^6$ in 0.02 ml). Group 3 animals received both UCB $CD133^+$ HSCs and MSCs at an equivalent total cell dose ($0.5x10^6$ + $0.5x10^6$ in 0.02 ml; total combined human cell dose $1x10^6$ in 0.04 ml). The animals

survived for 6 weeks. There were significant differences in the Doppler blood flow ratio measured at day 42 among the three conditions. Pair-wise comparison revealed significantly higher blood flow measured in animals injected with both MSCs and UCB 133^+ HSCs compared with those animals treated with MSCs alone ($p<0.05$). Taken together, these experiments show the synergy hematopoietic stem HSCs and MSCs in mediating murine angiogenesis responses to ischemic vessel injury

5. Other experiments conducted in my laboratory compared the effectiveness of UCB derived $CD133^+$ cells to BM CD 133+ cells, non-selected bone marrow-derived mononuclear cells (BM MNCs), and non-selected UCB MNCs at mediating neovascularization *in vivo* in a NOD/SCID mouse femoral ligation vascular injury model. As shown in the attached publication, Cytotherapy, 2010; 12: 67-78, we found: (i) $CD133^+$ cells selected from UCB exhibited robust vasculogenic functionality compared with BM CD133+ cells, BM MNCs, and UCB MNCs in response to ischemia; (ii) blood flow in NOD/SCID mice 4 weeks after femoral ligation was significantly higher in animals injected with UCB-derived $CD133^+$ cells compared to BM $CD133^+$ cells, UCB MNCs, BM MNCs and control at 28 days despite administration of a lower cell dose (0.5×10^6 $CD133^+$ versus 1.0×10^6 MNC), highlighting the benefit of using a selected cell population UCBs instead of heterogeneous MNCs or BM $CD133^+$ cells; and (iii) examination of human cell engraftment in the marrow was highest in mice receiving UCB derived selected $CD133^+$ cells compared to mice receiving BM $CD133^+$ cells, UCB MNCs, and BM MNCs.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Mary J. Laughlin M.D.

5/3/2011
Date: